BECKMAN COULTER
Life Sciences

Optimizing the Multisizer 4e Coulter Counter for use with small apertures

Introduction

Counting and sizing of cells/particles as small as 200 nm is possible with the Multisizer 4e Coulter Counter using high-resolution apertures (HI-RES, 30 UM; HI-RES, 20 UM and HI-RES, 10 UM). Beckman Coulter Life Sciences also offers Isoton II diluent, a standard aqueous electrolyte for many applications, which was used as a default electrolyte for the process described in this application note. Sea water, other aqueous and organic electrolytes can also be used for specific applications following the same steps presented here. Instructions for preparing other electrolytes, as well as recommended electrolytes for many materials, can be found in Appendices A-C of the Multisizer 4e Coulter Counter User's Manual.

This application note describes a method for successful testing using the Multisizer 4e Coulter Counter with apertures 30 µm and smaller. Additional information for changing and working with small apertures can be found in Chapter 4 of the instrument user's manual.

Here, we provide process steps for instrument setup and preparation, as well as sample measurement, based on a protocol that was successfully applied when working with small aperture tubes optimized for the lowest background noise and high signal-to-noise ratio performance.

Multisizer 4e Coulter Counter Operating program setup – User mode selection

1. To get the most of the instrument's control, we recommend the **Operator with Advanced Features** user mode. This requires a security setup (see Chapter 9 of the user's manual) to create an **Administrator** user and choosing **Low security (Figure 1)**. That provides access to the user mode dialog selection list. After the methods are established and saved for SOMs, any User Mode will be able to perform sample analyses successfully.

2. Select **Supervisor** user mode **(Figure 2a)**, then from the Supervisor tab select **Instrument Configuration** and check **Enable Dialog Help**. This will set up a help button throughout the workflow, which will take you to the section of the user's manual about the operation you want to perform **(Figure 2b)**.

3. From the Supervisor tab select **Change User Mode** and choose **Operator using advanced features** (Supervisor tab will change to Security with login and change user mode options).

Figure 1. Security setup.

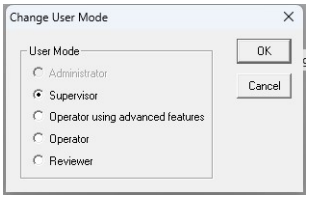

Figure 2a. Change User Mode.

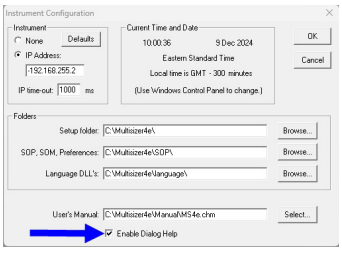

Figure 2b. Enable Dialog Help

4. Select **Enter Service Mode** from **Troubleshooting** (found in the **Run** menu). The **Service** tab will be created next to **Run**. We'll refer to this later. *(Service Mode must be enabled every time the program is opened after being closed).*

Steps for instrument setup and preparation

1. Remove the Electrolyte Jar from the Multisizer 4e instrument and thoroughly clean the jar, ensuring the final rinse is done with laboratory-grade DI water.

2. Fill the Electrolyte Jar with fresh Isoton II diluent.

3. For small aperture operation, the optimum position of the internal electrode is approximately 14 mm (≈ 1/2") up and away from the aperture's orifice, which is ≈ 7.5 cm from the top. The internal electrode can easily be repositioned by winding the electrode wire a few turns onto the Teflon filling tube and sliding the wrapped electrode upward **(Figure 3)**.

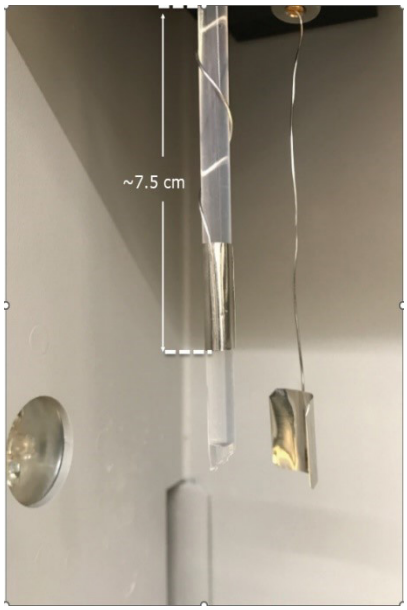

Figure 3. Internal electrode positioning.

4. Before installing the new aperture, rinse it with IPA or Ethanol *(Do not touch the orifice, and we recommend wearing particle-free gloves)*.

5. Turn ON the instrument and select **Change Aperture Tube Wizard** from the **Run** menu to install the new aperture. Follow it down and, at the **Set Current and Gain** step, use **Default** for 20 UM and 30 UM apertures. For 10 UM apertures, manually enter 300 µA for **Aperture current** and 2 for **Preamp Gain (Figure 4)**.[1] After setting current and gain, close the wizard and remove the electrolyte accuvette.

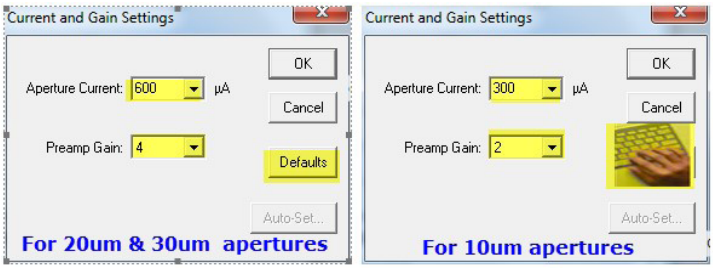

Figure 4. Current and Gain Settings for small apertures.

6. While the instrument warms up, fill an accuvette with hot water, add ≈ 1mL of Micro 90, mix it and place it on the sample platform.

[1] These settings have been optimized under more realistic environmental conditions than 10 µm aperture default settings—200 µA current and 4 gain—that were generated years ago in an ultra-quiet environment with higher blank counts expected.

7. From the **SERVICE** menu next to Run, select **Aperture Maintenance** and check only **Unblock Aperture** and **Repeat Sequence**, and then **Start (Figure 5)**. Cancel after 10 run cycles, cancel and close.

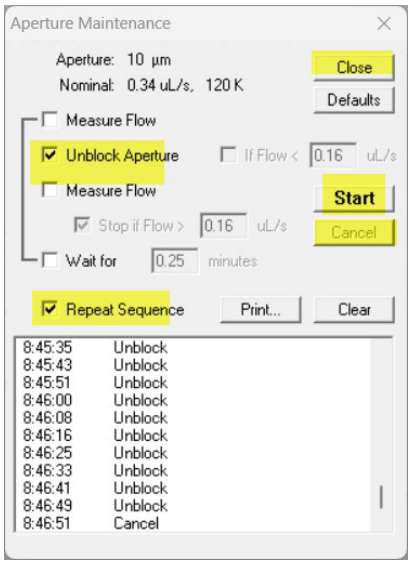

Figure 5. Aperture Maintenance.

8. Flush the aperture by pressing the icon at the bottom of the operating program.

9. Using a large beaker (e.g., 200 mL) with DI water, rinse the outside of the aperture tube and the electrode by completely immersing them in the water, gently swirling the fluid as you rinse down the aperture tube.

10. Use the Beckman Coulter Life Sciences Accuvette ST when performing small particle sampling. Ensure the new accuvettes and caps are rinsed thoroughly with lab-grade filtered DI water.

11. Place an Accuvette ST with ≈ 10 mL of fresh and clean electrolyte on the platform and perform preview until counts go down to near zero and cancel.

12. Flush the aperture.

13. Select **Instrument Start-up** from the **Run** menu, **click** on Default. If the waste tank is not full, uncheck **Empty the Waste Tank**, and start. *(These steps will be performed every day before using the instrument).*

Aperture Calibration and Verification

Beckman Coulter Life Sciences provides standard calibration controls specified for modal diameter calibration: L3 = 3 µm for the 30 µm aperture, and L2 for the 20 µm and 10 µm apertures. Any traceable standard can be used with mean diameter calibration as well. Suitable sizes for calibration standards are 10% - 20% of the aperture's diameter. While not imperative, for newly installed aperture tubes we recommend obtaining the average calibration factor, Kd, typically from 10 runs. Subsequently, single verification runs will suffice to confirm the aperture is ready for sample analysis.

1. Remove the Accuvette with the electrolyte, add a few drops of calibrator standard, cap it and gently mix it by rotating between your palms to avoid bubble formation, then replace on the platform.

2. Check the concentration level by clicking on **Preview**; 1% - 5% is OK (if adjustment is needed, add sample or electrolyte and mix again).

3. For calibration, use the **Kd Calib**. icon on the menu bar to select the appropriate options and 10 runs **(Figure 6)**. At the end of the last run, a new Kd value will be generated from the average of the 10 runs, and will prompt you to save it and update for subsequent runs **(Figure 7a)**. Accept and save the new Kd.

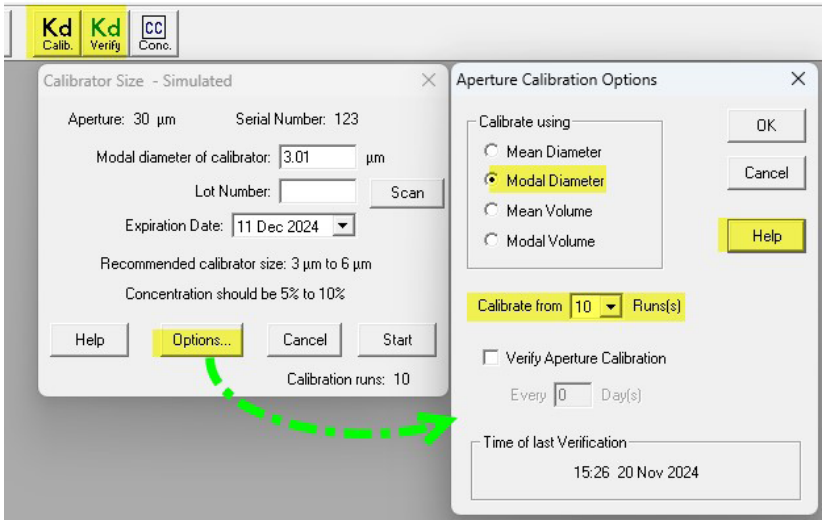

Figure 6. Calibration verification options: **Kd Calib**. for first-time calibration at installation, and **Kd Verify** for future verifications. For more information, **Help** will open the user's manual to the relevant topic.

4. In the future, only Verification runs will be performed using the **Kd Verify** icon after running Instrument Start-up. At the end of the run, you should see ***The calibration is OK*** message **(Figure 7b)**.

5. The ***instrument needs to be recalibrated*** message will appear when measured calibrator size change is greater than ± 3%. This could be caused by partial blockage or a dirty aperture, in which case recalibration might not be necessary. Instead, flush the aperture, perform **Unblock** once or twice, and run **Kd Verify** again. If it fails again, clean the aperture (follow steps 6-10 in the previous section) and perform the verification again.

6. Replace the cuvette with the calibrator suspension and run Verify again.

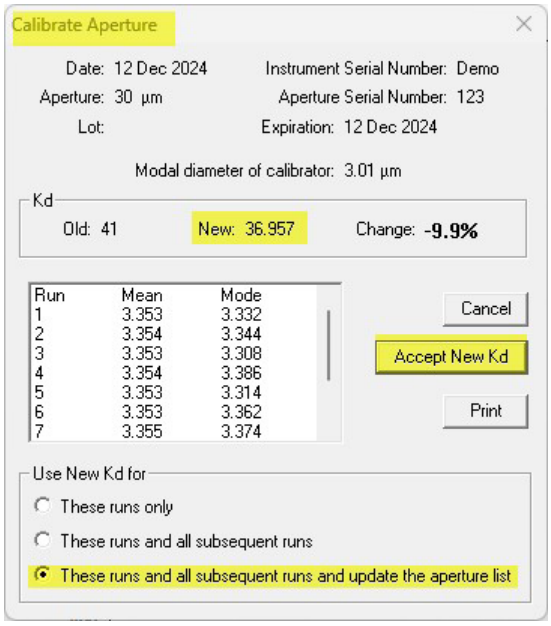

Figure 7a. Aperture Calibration result.

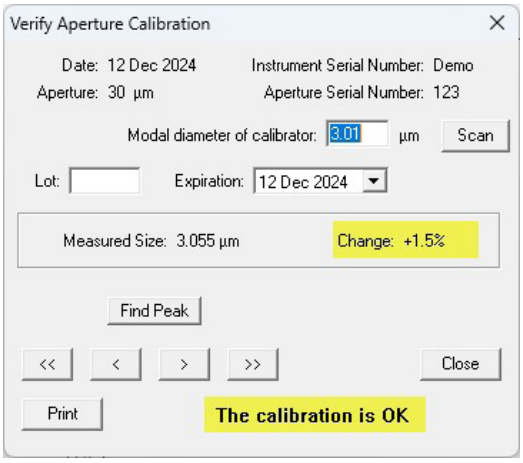

Figure 7b. Verify Aperture Calibration result.

7. Have an Accuvette ready with clean electrolyte—capped to protect from dust.

8. Remove the accuvettes with the standard used for calibration/verification and rinse the aperture and the external electrode with DI water or clean electrolyte, and **perform the next step immediately** to prevent the aperture from drying, which will create noise.

9. Place the accuvette with clean electrolyte on the platform, press Preview, and watch concentration counts drop to zero (or near zero) when all beads and DI water from the aperture are inside the tube, then cancel.

10. Flush the aperture tube by clicking on the Flush button.

Process steps for running samples

1. Run 3 blanks using the same **Control Mode** that will be used in your SOM when running samples, e.g., Volumetric or Time control mode: flush after each run, etc. **(Figure 8)**.

2. Setting for 3 runs is recommended for most samples, but some samples are ***not*** suitable, for different reasons, such as samples with broad size distribution or a tendency to stick to the aperture. In such cases, it is best to acquire one-by-one, with a flush at the end of each run, or unblock between runs to redisperse particles that gather at the aperture via inertia. The second option should be used when error messages appear on a second or third run after a *successful* first run, or when the overlay of 3 consecutive runs shows large differences on counts. Here, we cover only specific parts of the SOM. Complete instructions for SOM set up are in chapter 5 of the User's Manual.

3. Open **Edit SOM**. In the **Control Mode** tab:

   - Select time or volumetric (typical volumes 75 µL, 50 µL or 10 µL for 30, 20 and 10 UM apertures, respectively, that last ≈ 30 s).
   - Click on the corresponding buttons and set **Waste tank** and **Fill, Flush & Drain** to defaults.
   - Click on **Resistance** and ensure both **Enable** options are not checked.
   - Click on **Sample delivery**; to help ensure the highest accuracy, set pre-flow stabilizing time to 10 seconds, and total stabilizing time to 15 seconds **(Figure 9)**.

4. When running one run at a time for the reasons above, perform **Unblock** on completion of each run to ensure there is no buildup of material at the aperture inlet. **Unblock** will disperse particles that gathered at the aperture by inertia after a run.

5. Some samples, such as proteins, may require cleaning between runs with a 5% solution of Micro 90 Cleaner. This is because of the tendency of proteins to stick to the aperture surface and create noise. Use a squirt bottle of DI water to spray the aperture after using the Micro 90 Cleaner.

6. In general, it is good practice to clean with Micro 90 solution between samples and at the end of the last sample to maintain the aperture in optimal condition.

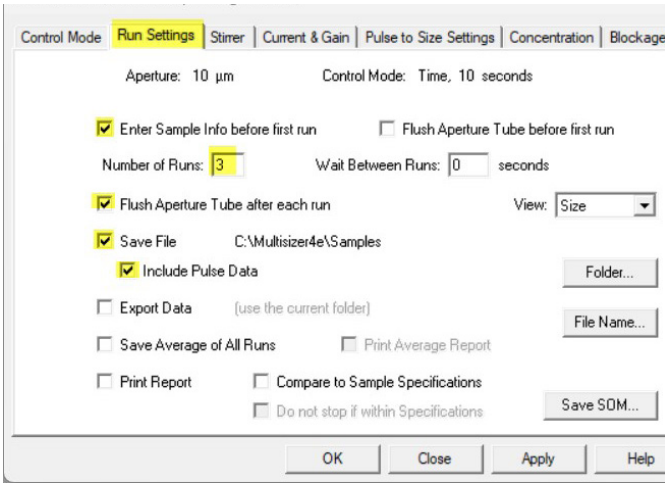

Figure 8. Run Settings.

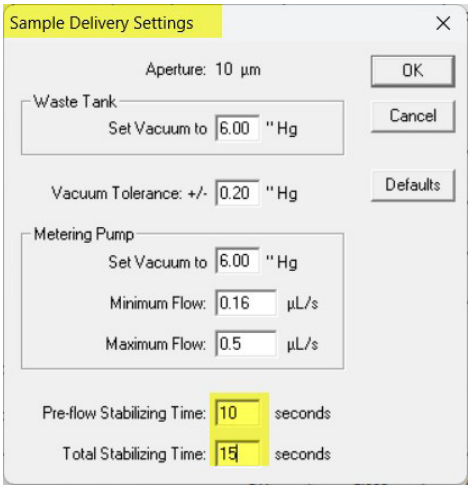

Figure 9. Total Stabilizing Time.

Figure 10 shows examples of clean blanks with the 10 µm aperture and Isoton II diluent under these conditions: 1) Unfiltered collected from the spout on the 10 L container; 2) Unfiltered from a settling jar; 3) Filtered with 0.2 µm syringe filter; and 4) Filtered with 0.2 & 0.1 µm syringe filters in series.

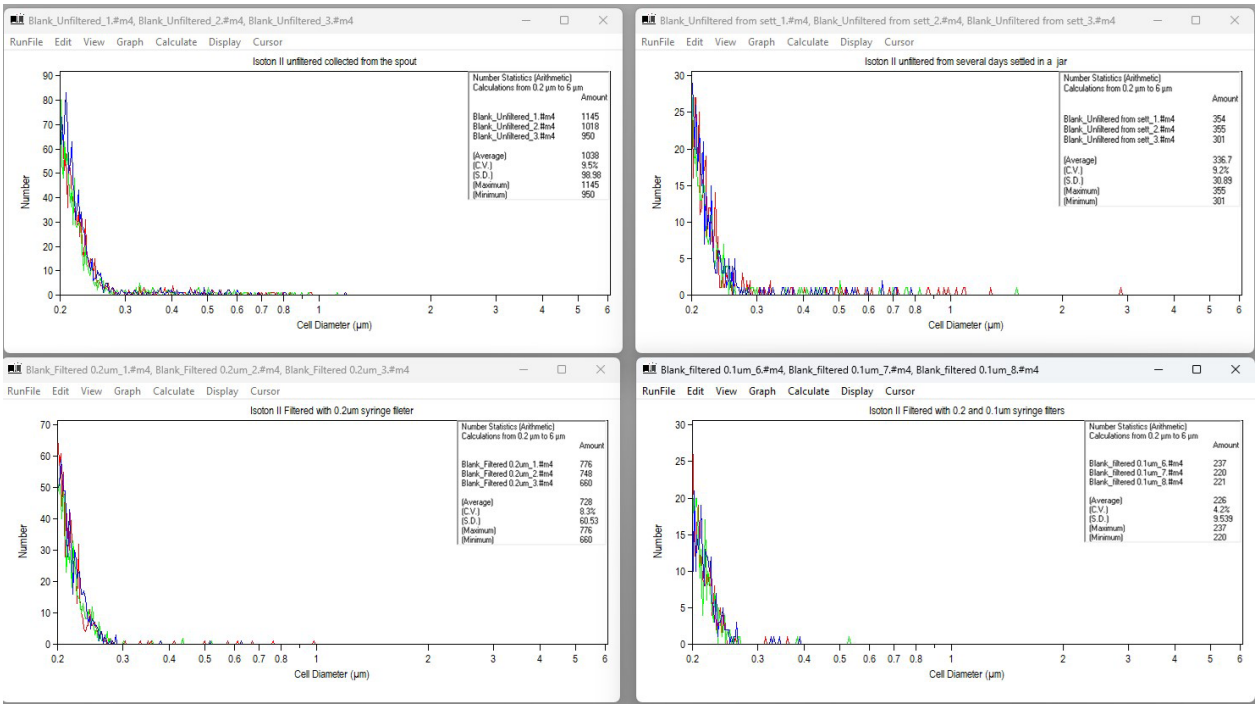

Figure 10. Isoton II filtered or unfiltered blanks <2200 counts/10 µL runs with 10 µm aperture.

For runs shown in Figure 10, filtering the electrolyte to 0.1 µm helped to reduce blank counts from ≈ 1000/10 µL runs with unfiltered Isoton II, down to ≈ 200/10 µL runs. Some filtering instructions and recommendations are discussed at the end of this application note.

Figure 11 shows sample runs of cancer treatment drugs encapsulated in liposomes or lipid suspensions for IV delivery, performed with a 10 µm aperture using volumetric control at 10 µL.

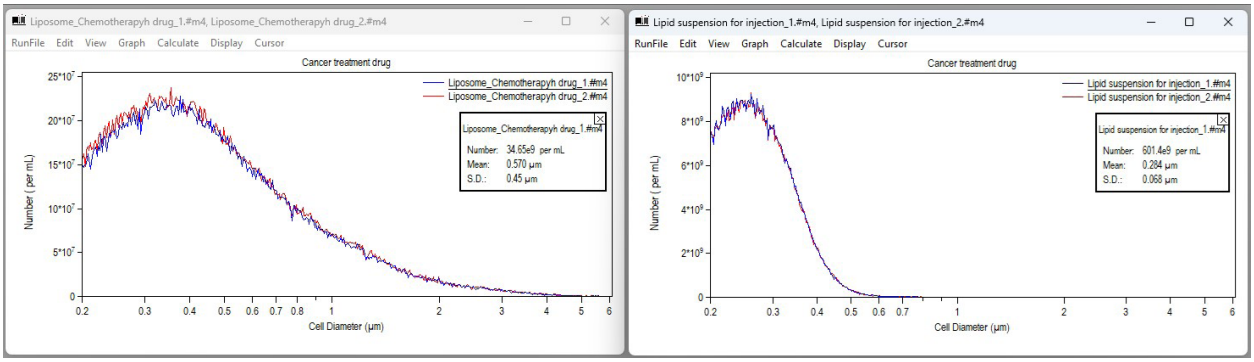

Figure 11. Cancer treatment drugs encapsulated in liposomes or lipid suspensions for IV delivery.

Filtering Isoton and electrolyte solutions

For easy and accurate dispensing of Isoton II electrolyte solution, a graduated dispenser that fits different bottles was used. For convenience, a 2 L bottle of IsoFlow Sheath Fluid, PN 8547008, was used.

The dispenser has a nozzle to which a syringe filter or series of syringe filters can be fitted to remove any stray particles and ensure the cleanest possible electrolyte solution for use with the sample.

Beckman Coulter Part Number	Description
8320309	Adjustable Volume Pipette Dispenser
8547008	IsoFlow Sheath Fluid container
C96980*	Isoton II diluent 10L (Americas)
8448011*	Isoton II diluent 20L (Europe/METAI)
8546719*	Isoton II diluent 10L (East Asia/Australia)
	PALL Acrodisc® Syringe Filters
4611	0.1µm, 25 mm
4612	0.2 µm, 25mm

*Part number varies depending on the region.

Figure 12. 8320309 Adjustable Volume Pipette Dispenser.

Figure 13. 4611 & 4612 syringe filters.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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